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Customized impedance spectroscopy device as possible sensor platform for biosensor applications

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A new impedance spectroscopy unit is developed, fully customized to become a vital part of label free biosensor arrays with possible applications in DNA and protein sensing. Test measurements are conducted to explore the accuracy and specificity of the system, both under electronic lab conditions as well as under wet cell conditions with synthetic-diamond based sensor electrodes. The impedance of seven resistors was monitored for 17 h and a maximum error <0.02% was found. Furthermore, the impedance of PBS at different concentrations was monitored for 60 min per concentration and a different impedance for each concentration was detected. The impedance is also monitored for NaOH, PBS and nuclease free water at different temperatures with a total duration of 60 min per fluid. Systematically different impedances for each temperature per fluid were found and the temperature coefficients were determined. All test measurements lead to results well within specification.

1 Introduction Impedance spectroscopy is an upcoming technique in the field of biomedical research [1]. This technique has already been used with success for monitoring DNA hybridization and the detection of single nucleotide polymorphisms [2], recognition of nicotine by means of molecular imprinted polymers [3] and the detection of C-reactive protein (CRP) [4]. The impedance-based detection offers the possibility of device miniaturization and an electronic read-out. After it was clear that it was possible to monitor hybridization and detect single nucleotide polymorphisms it was time to explore the possibilities to increase measurement speed, allowing for semi-simultaneous detection of multiple types of receptors with their corresponding target molecules [5]. Therefore a new unit is developed, based on a single chip developed by Analog Devices [6]. When Analog Devices presented a single chip solution for impedance measurements in 2005, it meant a revolution. Traditionally, it was an extreme design challenge to come up with a scheme to measure impedance. The only way it could be done was by using up to nine different integrated circuit chips, each with its own power provisions, separate resistors and capacitors. The design included an A/D converter, temperature sensors, different amplifiers *etc*. All these components had to be configured to operate at the same level and all the signal paths had to be investigated, not to influence the measurement by crosstalk effects. The integration in a single chip provides the possibility of miniaturizing the hardware which is much better suited for point of care diagnostics (POC) and eventually lab-on chips. It also means a huge saving in surface area required for an impedance measurement setup and an enormous saving in design and testing time of the setup. Another note worth mentioning is that the cost of this device is 50% less than the cost of a multichip solution. The impedance chip is able to measure frequencies between 100 Hz and 100 kHz, with an accuracy of at least 0.5% [7]. The impedances measurable are in the range of 100 Ω and 10 MΩ. These overall numbers and facts are impressive, but when incorporating the chip in a

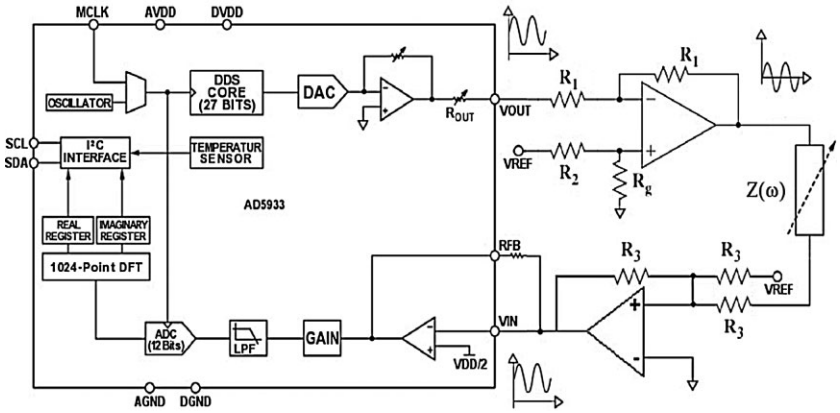

Figure 1 Circuit to remove the bias voltage in front of the sample under test and put it back on the signal behind the sample.

design to measure the impedance of biological samples, one is stopped in their tracks. The Analog Devices AD5933 uses a direct current signal, with a superimposed alternating current signal, with a variable frequency [8]. This DC bias of $^2/_3$ voltage drain drain (VDD) can ruin a measurement because the ions of the analyte are forced toward one electrode. This results in deterioration of the electrodes due to corrosion or even destruction of the sensor surface because of the occurrence of hydrolysis. In order to do the biological measurements, it was necessary to strip the DC voltage or at least make the bias user controllable [9]. The AD5933, unfortunately, did not provide the option to remove a bias voltage, as it is aimed primarily for automotive and fuel/battery cell monitoring purposes. So a circuit had to be designed to remove the bias voltage in front of the sample under test and put it back on the signal behind the sample [10]. This circuit is schematically represented in Fig. 1. This work presents the characterization of the new developed impedance spectroscopy unit based on this chip. Measurements have been conducted to proof the accuracy of the unit, not only on electrical resistors, but also on phosphate-buffered saline solution (PBS), NaOH and nuclease free water, at different temperatures and concentrations. The measurements were successful; it was possible to distinguish even slight differences in concentration and temperature of the fluid and an accuracy of $<0.02\%$ is proven.

2 Experimental To investigate whether or not the developed unit is applicable for the biological detection, test measurements were performed to prove the use of this chip for biosensor purposes.

2.1 Accuracy and durability test Accuracy of the unit was tested by means of measuring the impedance of seven resistors with a value of 36, 20, 12, 11, 4.3, 2.8, and 2.7 kΩ. The impedance was monitored for 17 h at frequencies ranging from 100 Hz to 100 kHz, build up logarithmically with 10 frequencies per decade and a scanning speed of 5.3 s per frequency sweep. Because the impedance of an ideal resistor is purely real, the impedance of the chosen resistors should not change over time [11]. The maximum error is calculated with formula (1):

$$\text{maximum error} = \frac{|Z|_{\max} - |Z|_{\min}}{|Z|_{\text{real}}} 100. \quad (1)$$

The resistors had a common copper ground and were measured sequentially by means of an analog multiplexer with an internal resistance of 0.03 Ω, integrated in the impedance unit. In Fig. 2, the setup of this experiment is schematically represented.

2.2 Impedance of electrolytes at various concentrations and temperatures Given the fact that the biosensor measurements are performed in a liquid cell, the influence of ionic solutions at different temperatures had to be investigated [12]. The solutions used in the protocol are PBS as a common buffer, NaOH, frequently used to denaturate DNA, and nuclease free water to dissolve DNA

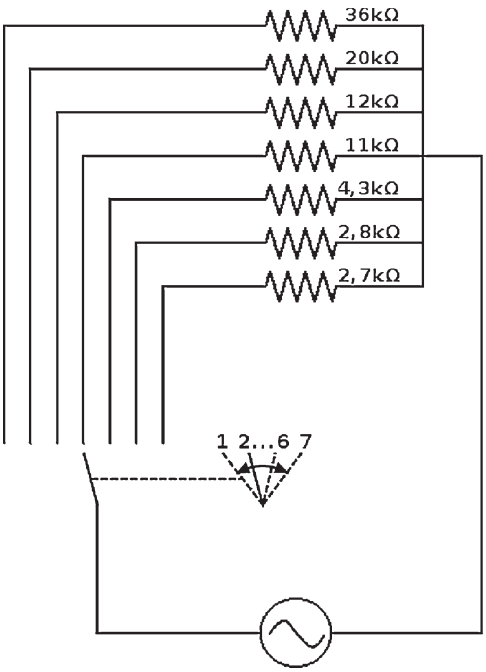

Figure 2 Schematic representation of experimental setup to determine durability and accuracy of the impedance unit.

[2]. For this study, a 1 L stock of 10× PBS was prepared by dissolving 80 g NaCl, 2 g KCl, 14.4 g Na_2HPO_4, and 2.4 g KH_2PO_4 in 0.8 L of distilled water, and topping up to 1 L. Nuclease free water, containing diethylpyrocarbonate (DEPC) to preserve DNA, was bought at Ambion Applied Biosystems (Japan) and 1 M NaOH solution was purchased at VWR (Belgium). First, the impedance was measured for PBS at different concentrations (10× PBS, 1× PBS, 0.1× PBS, and 0.01× PBS all with a volume of 140 μL), starting at the lowest concentration, in a frequency range of 100 Hz to 100 kHz built up logarithmically with 10 frequencies per decade and a scanning speed of 5.3 s per sweep. The conductivity and therefore impedance should be linearly dependent on the concentration. However, this is only true in the lower concentration range where the tendency to form ion atmospheres and dipole states is negligible. The solutions are assumed to have a strong temperature coefficient, ranging from 1.4 to 2% per °C, because the viscosity of the liquid and the resulting frictional force on a migrating ion decreases with increase in temperature [13]. In Fig. 3a the setup is represented which was used to measure the impedance of PBS at different concentrations. A nanocrystalline diamond sample (NCD), functioning as a working electrode, was mounted on a copper back contact using silver paste. Diamond has a high thermal conductivity, is transparent in a wide region of wavelengths, is chemically inert, has a wide electrochemical window, and can be made semiconducting by chemical doping [14]. Diamond is biocompatible allowing eventually for *in vivo* applications and, moreover, it is suited for covalent attachment of biochemical receptors [15–17]. A rubber O-ring with an inner diameter of 7 mm and a teflon lid containing a circular opening of equal size was pressed onto the sample to create a reaction well above the NCD. The well was filled with 140 μL of reaction fluid. A gold wire, placed 1 mm above the NCD surface in contact with the reaction fluid, was used as a counter-electrode. The working and counter-electrode were connected to the impedance analyzer via 50 Ω coax-cables. All concentrations were measured in the same well to prevent crosstalk between different wells. This setup was put in an oven (Binder E28) at an ambient temperature of 37 °C and functioning as a Faraday cage. An identical setup, represented in Fig. 3b, was used to measure the impedance of all solutions at different temperatures with a frequency range of 100 Hz to 100 kHz, again with a volume of 140 μL. The setup was put in the oven but now it was exposed to different temperatures, ranging from 37 to 50 °C.

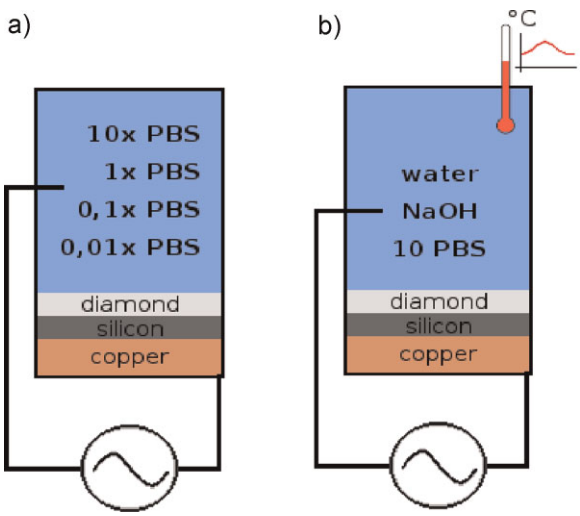

Figure 3 (online colour at: www.pss-a.com) Experimental setup for impedemetrical differences at various concentrations of PBS (a), and various temperatures of nuclease free water, NaOH and PBS (b).

3 Results

3.1 Accuracy and durability test Sequential measurements were performed using seven electrical resistors. In accordance with the specifications from the AD5933 impedance chip the maximum error is <0.02% for all resistors at all frequencies [7]. In Fig. 4, the Bode plot is shown for the sequential measurements showing the impedance in the total frequency range for seven resistors, monitored for 17 h. The absolute error however (maximum measured value minus minimum measured value), does increase with increase in resistor value and was determined 6 Ω for the 36 kΩ resistor, 4 Ω for the 20 kΩ resistor, 2.3 Ω for the 12 kΩ resistor, 2.2 Ω for the 11 kΩ resistor, <1 Ω for the 4.3 kΩ resistor, <1 Ω for the 2.8 kΩ resistor and <1 Ω for the 2.7 kΩ resistor. It also became apparent that the impedance of all resistors decreased after 11 kHz, due to the capacitor effects in the electrical resistors.

3.2 Impedance studies on electrolytes The impedance is measured for PBS at different concentrations (10× PBS, 1× PBS, 0.1× PBS, and 0.01× PBS all with a volume of 140 μL) in a frequency range of 100 Hz to 100 kHz and an ambient temperature of 37 °C. All concentrations were measured in the same well to prevent inter-well confounding. In Fig. 5a the real and imaginary parts of the impedance are

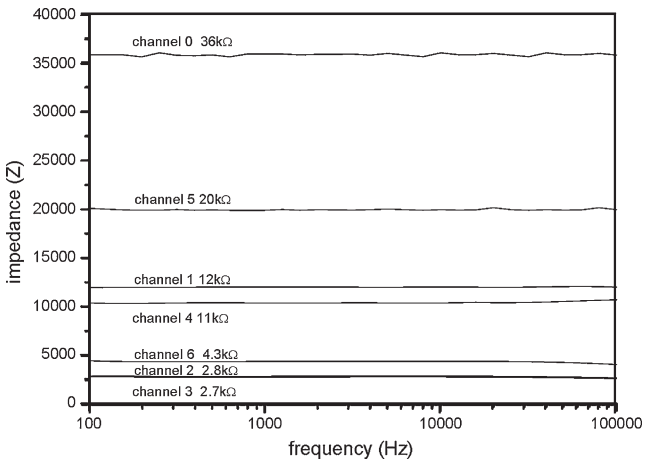

Figure 4 Bode plot of a sequential measurement showing the impedance in the total frequency range for seven resistors, monitored for 17 h.

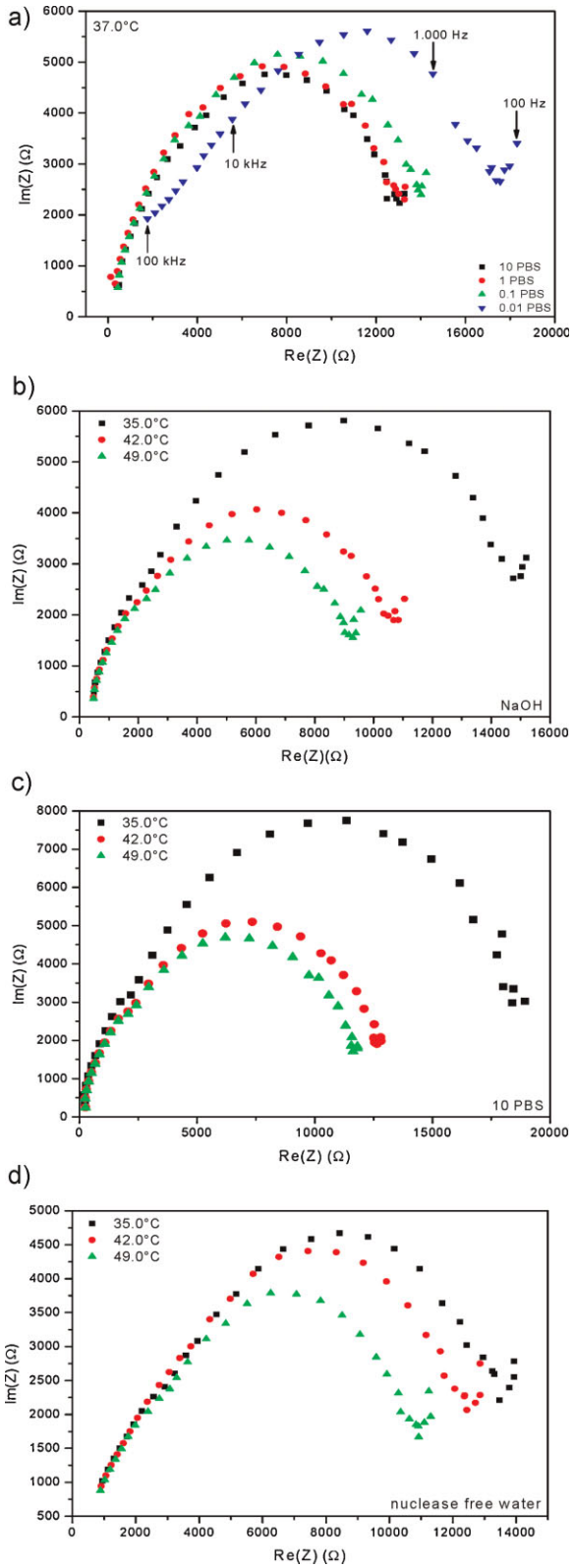

Figure 5 (online colour at: www.pss-a.com) Nyquist plot showing the real and imaginary part of the impedance for 10× PBS, 1× PBS, 0.1× PBS, and 0.01× PBS (a), for NaOH at 35.0, 42.0, and 49.0 °C (b), for 10× PBS at 35.0, 42.0, and 49.0 °C (c) and for nuclease free water at 35.0, 42.0, and 49.0 °C (d).

represented in a Nyquist plot. It can be observed that all concentrations show different plots and sequentially the lowest concentration shows the highest impedance and *vice versa*. Secondly, the temperature dependence of PBS, NaOH, and nuclease free water was measured in a frequency range of 100 Hz to 100 kHz, with a volume of 140 μL, again the setup was put in an oven but now it was exposed to different temperatures ranging from 35 to 50 °C. In Figs. 5b, c and d the real and imaginary parts of sequentially NaOH, 10× PBS and nuclease free water are represented in Nyquist plots for three different temperatures. It can be seen for all solutions that the greatest difference occurs in the lower frequency ranges. Furthermore it can be seen in Figs. 5b and 5c that for NaOH and 10× PBS the difference in impedance is the greatest in the temperature leap from 35 to 42 °C, both in real and imaginary part, unlike nuclease free water where the greatest difference is found between 42 and 49 °C as shown in Fig. 5d. To investigate the linearity of this process, the impedance of NaOH, 10× PBS, and nuclease free water, when increasing the temperature, was linearly fitted for 100 Hz, 1, 10, and 100 kHz providing a Coefficient of determination (R^2) as a measure for linearity. In Table 1 it becomes apparent that the impedance decrease per increase in °C diminishes with rising frequency. However a decrease in R^2 is observed above 11 kHz, probably caused by the capacitor effects described earlier.

4 Discussion This article demonstrates a newly developed impedance spectroscopy unit, customized to become part of a label-free, diamond-based DNA or protein sensor array. Accuracy and robustness of the system under electronic lab conditions as well as under wet cell conditions is shown. It is proven that the maximum error of the system is <0.02% under electronic lab conditions, but an increase in absolute error was monitored with increase in resistance. At frequencies below 11 kHz, the total impedance was equal to the resistor value. However, above these frequencies the impedance decreased below this resistor value. The impedance decrease at high resistor (Z_R) values (11–36 kΩ) is caused by a parallel parasitic capacitance of the sample. The impedance (Z_C) of this capacitor is inverse proportional to the frequency, as shown in formula (2):

$$Z_C = \frac{1}{2\pi f C}. \tag{2}$$

At lower frequencies this Z_C is too high to have an effect on the total impedance (Z), but at higher frequencies Z_C becomes low enough to cause a significant decrease in Z. This is illustrated in formula (3):

$$Z = \frac{Z_R Z_C}{Z_R + Z_C}. \tag{3}$$

Furthermore, if Z_R has a higher value, a decrease in Z_C is more apparent in proportion to the total Z than at lower Z_R values. This caused the slope of the graph to be much steeper at higher resistor values as is seen in Fig. 4. The calculated

Table 1 Impedance decrease (%) per increase in °C for NaOH, 10× PBS and nuclease free water for 100 Hz, 1, 10, and 100 kHz.

	frequencies			
fluid	100 Hz	1 kHz	10 kHz	100 kHz
NaOH	3.1% ($R^2 = 0.874$)	2.6% ($R^2 = 0.879$)	0.7% ($R^2 = 0.759$)	0.6% ($R^2 = 0.830$)
10× PBS	3.1% ($R^2 = 0.780$)	2.5% ($R^2 = 0.789$)	0.8% ($R^2 = 0.790$)	0.5% ($R^2 = 0.691$)
nuclease free water	1.7% ($R^2 = 0.998$)	1.3% ($R^2 = 0.905$)	1.0% ($R^2 = 0.981$)	0.7% ($R^2 = 0.951$)

accuracy is high enough to recognize *e.g.*, DNA hybridization with an impedance drop of 20% at a frequency of 1 kHz as described in Ref. [2]. The ability is shown to detect differences in concentration of PBS. The accuracy of this detection is also high enough to detect differences in concentrations which may occur when (re)filling the wells with PBS or ssDNA dissolved in nuclease free water. Also, the accuracy of detection of impedance changes due to temperature effects is sufficient. The fact that the impedance decrease per °C diminishes with rising frequency might be attributed to the fact that the impedance of a fluid is more apparent in the higher frequency ranges in opposition to the impedance change of the solid parts of the setup (NCD, copper) which becomes less pronounced in the higher frequency ranges. In the future the frequency range will be extended from 10 Hz to at least 1 MHz, build up logarithmically with 10 frequencies per decade. Furthermore the setup will be scaled down. Reaction wells, which need great volumes and are therefore more vulnerable to temperature bias, will no longer be a necessity. A reaction chip is under development which enables an eight channel measurement over a surface of 1 cm^2. Using this chip will make great volumes, long wires *etc*. redundant and hence improve the accuracy and specificity of the system.

5 Conclusions According to the measurements described above the developed impedance spectroscopy unit is considered to be robust and accurate. Long-term measurements give accurate results and it is proven that the unit not only functions under electronic lab conditions, but also under fluid cell conditions. It is expected that the system is employable as vital part of a label free affinity biosensor system. However, the system still leaves room for improvement. In the future the frequency range will be extended from 10 Hz to 1 MHz so that protein–antibody recognition is possible (at 10 Hz) [18] and DNA denaturation can be detected (at 1 MHz) [2]. Furthermore the setup as shown in Figs. 3a and b will be miniaturized. Reaction wells which need great volumes will no longer be present in the renewed setup. The developed system is already prepared for this down scaling and can directly be deployed for these measurements.

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